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Quagga Mussel Invasion of Western Europe

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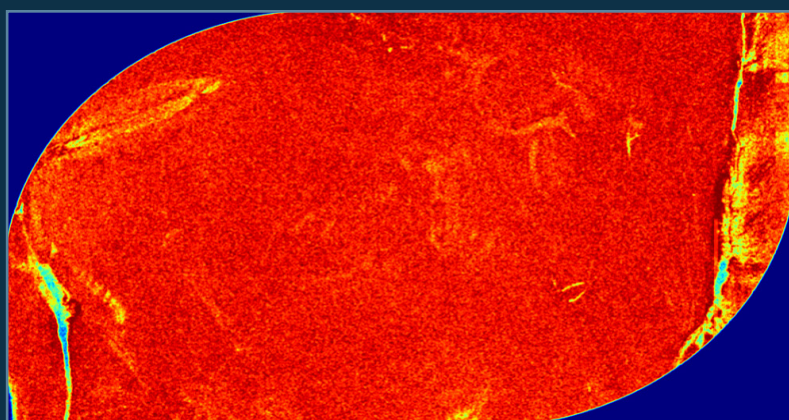
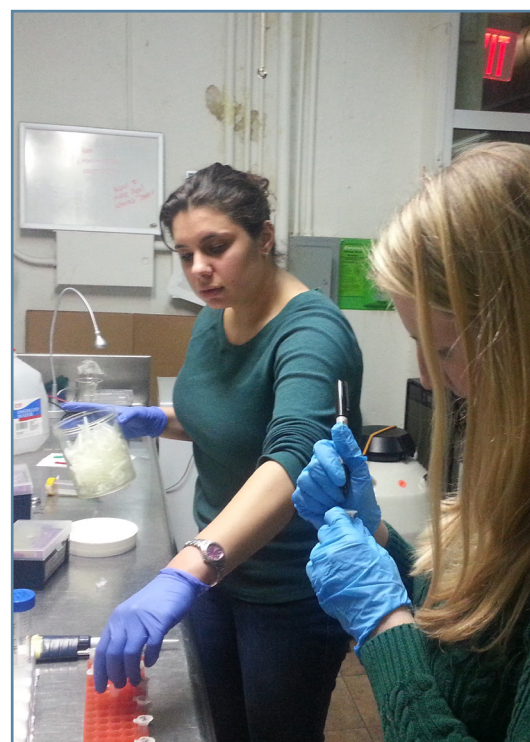
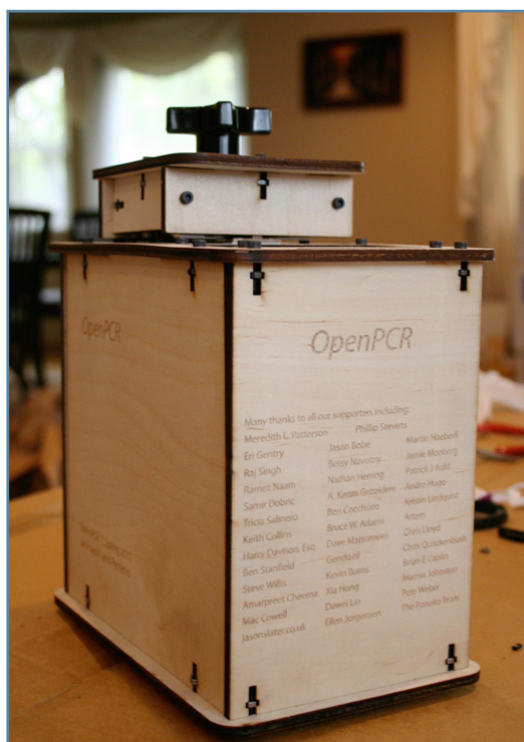
New Partnership Supports iBOL

In December 2013, Life Technologies Corporation, a leader in DNA sequencing technology, announced a partnership with the Canadian Centre for DNA Barcoding (CCDB). This collaboration will support iBOL-led projects, specifically the study of biodiversity patterns in the very diverse areas of Central and South America as well as the expansion of the insect DNA barcode reference library through the analysis of Malaise trap samples from around the world. In addition, through this partnership, next generation sequencing applications in DNA barcoding will be further developed with the aim of improving our understanding of global biodiversity.



Barcoding For The World

DIY initiatives broaden the reach of DNA barcoding



Virtual Ion 316™ Chip loading heat map generated by the Personal Genome Machine™ System, demonstrating percent loading across the physical surface.

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DIY Barcoding

Editorial feature highlights the past success and future potential of Do-It-Yourself programs

The worldwide community of “amateur biologists”, biohackers, citizen scientists, or otherwise “non-institutional scientists” want to push the envelope of biological technology. They want to, in general, increase the power of an individual to understand biological systems – “to understand things” – and to prototype biological designs – “to build things”. They ask why the tools we use as biological professionals – protocols and equipment and organisms and genes – are the way they are, and perhaps not simpler, less expensive, or just easier.

We increasingly rely on citizen scientists to collect data for our research, in particular the network of biological enthusiasts who provide much of our knowledge on the changing distribution of species. Many of these people are interested in using technology to maximize the value of the data they collect, but don’t have the financial or technological resources to make full use of the opportunities technology provides.

In 2008 Mackenzie Cowell and Jason Bobe created an organization dedicated to making biology an accessible pursuit for amateur biologists and biology professionals. In so-called “biohacklabs” at DIYbio, amateur biologists could come together to create projects, not just for fun, but also to improve their knowledge or engage in existing projects contributing to current research efforts.

This idea took off quickly and the DIYbio network currently consists of 29 groups in 14 countries. One of those is LaPaillasse, led by a PhD student in Synthetic Biology. This first French community lab for biotech is a real lab connected to /tmp/lab, a hacker space in Vitry sur Seine for people doing creative things with technology, culture and arts as an online community. The goal is to get together people from the most varied horizons, making projects together and sparking new ideas for the 21st century. Various people are

developing new ideas, new projects, new arts, bridging the digital divide, helping people to grasp technology in a creative manner, communicating an open vision of the world, empowering people to develop their project with new technologies.

Much of the enthusiasm for do-it-yourself biology arises from the concept that small, entrepreneurial businesses, not large global corporations, are often the ones that introduce new ideas and technologies to the world. It is the large, global corporations that subsequently commercialize these innovations. The way the biohackers see it, do-it-yourself biology is essentially an enormous number of small companies doing free research and development. They want to revise the notion that you must be an academic with an advanced degree to make any significant contribution to the biology community.

DNA barcoding provides an outstanding basis for amateur biologists because its workflows are simple enough that amateurs can gain exposure to all aspects of the analytical chain from specimen collection to data interpretation without the need to study an insurmountable amount of background information. It comes as no surprise that DNA barcoding is omnipresent in the project lists of the various DIY labs that keep popping up everywhere in the world.

We’ve decided to make space in the Barcode Bulletin for a DIY Barcoding corner. Every future issue will have at least one contribution updating you on what is going on in the amateur biologist community. It is our hope to facilitate communication and interaction between professional and amateur scientists interested in biodiversity. We will start with an article about work done at Genspace, a non-profit community laboratory in New York City (page 11).

Written by: Dirk Steinke

Identifying Medicinal Plant Roots in Trade:

DNA barcoding in Morocco

In marketplaces around the world, from Buenos Aires, to Benin, to Baghdad, people trade medicinal plant products. These herbal medicines are often culturally important and their use dates back many generations. Local demand for herbals has grown with increasing urbanization and welfare, and plant species that were available in the past may now have become scarce due to over-harvesting or degradation of natural habitats. As species become rare, people may also opt to substitute similar alternatives for the original species, but incentives for adulteration emerge as well. Understanding what species are traded today can help us to monitor trade in threatened and endangered species and to detect potentially harmful adulteration with toxic species. Plant products such as bark, roots and powders are hard to identify, and DNA barcoding has helped us to shed light on this trade.

Marrakech is a crossroads of biological and cultural diversity, situated at the foot of the High Atlas range. The medina of Marrakech has a bustling market full of herbalist shops with jars of roots and piles of fragrant spices, wholesalers with burlap sacks from across Northern Africa and ambulatory traders with freshly picked spices and produce from the mountains. The Arabs and Amazigh have been trading plants here for ages and collectors, middlemen, retailers and consumers have abundant knowledge of herbal remedies, spices and talismans.



Extensive research by the Global Diversity Foundation (GDF) has found that over 300 species of plants and 80 species of animals are currently commercialized in southern Morocco. Identification of roots and barks has relied on matching of vernacular names to traditional pharmacopoeias and in many cases species identity has been far from certain. In collaboration with GDF, we used DNA barcoding to investigate which medicinal roots are really commercialized. A regional reference database was created of putative species and their sister taxa and sequence data from both plastid (*matK*, *psbA-trnH*, and *rpoCl*) and nuclear (nrITS) markers. The reference database and query sequences were submitted to Barcode of Life Datasystems (BOLD), and BLAST was used to match query sequences from roots purchased in the medina. Out of 83 samples, 56% were identified to species level and another 36% to genus level. In 18% of the cases, identification differed from hypotheses based on vernacular names. In a follow-up study into four complexes of medicinal root products with high morphological variety, 47 roots were sampled and yielded 91% species level identifications. Here each complex comprised more than one species, but none of the ones previously asserted based on previous literature.

Our study shows that the majority of the traded roots belong to species that are common and not known to be endangered. Nevertheless, endemic plant species are commercialized in Marrakech and species adulteration is common. A significant conclusion from our studies is that DNA barcoding is a powerful tool for identification of unknown samples as long as comprehensive reference data are available. It also underlines the importance of DNA barcoding for monitoring of trade in endangered plant species, as identifications based on folk taxonomy can vary widely in accuracy.

Funded by the Swedish Science Council – Swedish Research Links program and Marie Curie Initial Training Network – MedPlant.

Written by: Hugo de Boer and Anneleen Kool

Barcoding Blackfly Museum Specimens:

Facilitating the identification and monitoring of a “bloody” important group

Blackflies comprise an estimated 2,154 valid species. In the majority of blackfly species, the female requires a blood meal for egg maturation, and it is this requirement that makes them medically important. Their bite causes acute itchiness and irritation to the skin. The most important human parasites transmitted by blackflies are the nematodes *Onchocerca volvulus* (pictured on right), the causative agent of river blindness, and *Mansonella ozzardii*, which causes Mansonelliasis. They also transmit pathogens to domestic livestock, resulting in increased mortality, reduced weight, decreased milk production, and malnutrition. Not all blackflies are bad however - many act as “keystone” organisms in freshwater ecosystems because their larvae can filter dissolved organic matter, making it available to other organisms. They can also act as bio-indicators due to their sensitivity to organic and inorganic pollutants.

Given the social, medical, economic, and environmental significance of blackflies, it's not a surprise that their identification and monitoring is of paramount importance. Blackflies are therefore a target group of iBOL, which aims to assemble a DNA barcode reference library for all recognized blackfly species. Many of these species can simply be caught in the wild, though that is not always possible if the species are rare (or possibly extinct) or living in difficult to access places. Fortunately, no matter how difficult to collect a species is, if it's a recognized species it must exist in a formal collection somewhere.



Applying DNA barcoding to museum collections poses its own challenges ([shown in the last Bulletin issue](#)), namely due to the degraded nature of the specimen's DNA and also the size of specimens. Standard barcoding techniques will often not work on museum

specimens, and in the case of blackflies it was discovered that a blackfly specific mutation in the COI barcode region further complicated matters. The ability to barcode blackfly museum specimens is critical for the completion of the blackfly reference library, so a solution was needed.

In our study, examination of blackfly COI gene sequences allowed for the design of new primers that should overcome the mutation issue. DNA was extracted from the legs of 271 pinned museum blackflies and subject to standard museum barcoding techniques using the new primers. The results were positive, with the new primers performing significantly better than those previously available. In the end, sequence data were recovered from 80% of the test specimens - an excellent success rate for museum specimens. Analysis of the sequence data showed that even short sequences can be useful for species discrimination, a finding that will be very useful for the construction of the blackfly reference library.

Even in this relatively small dataset, several potentially cryptic species (a species that turns out to actually be multiple species) were uncovered, highlighting our lack of understanding of blackfly taxonomy and the need for further study. Such studies should be significantly easier now that the previously untapped blackfly resources housed in museums around the world can take full advantage of DNA barcoding.

Written by: Luis M. Hernández-Triana and Sean Prosser

Images by: Luis M. Hernández-Triana



Drawer with blackfly specimens at the Natural History Museum.

Dissecting the Food Webs of the Far North:

DNA barcoding changes our view on who eats whom

All nature is structured as food webs, where different types of predators feed on different types of prey. Figuring out not only who feeds on whom but how often this happens is the basis for understanding how nature works. With this we will be able to retrace e.g. what predator species may affect what prey, how different prey species may indirectly affect each other through a shared predator – or how a new species in the web may affect the others. We may also guess at how stable the web will be in the face of disturbance. Yet, measuring food webs is hard, as we will rarely encounter one species feeding on another – and even in cases where we do, some predator-prey interactions will typically remain hidden.

As an earlier remedy to these problems, much of what we know about food webs has been built on a particular type of interaction: that among parasitoids and their prey. Here, the parasitoid will develop inside of a host, typically killing it in the process. Taking advantage of this feeding mode, researchers have thus reconstructed parasitoid-host webs by growing hosts individually until either the adult host or its adult parasitoid hatch. If the individual emerging is a parasitoid, we will know whom it ate.

A remaining problem is that many hosts will die in the process. **Now a new study** circumvents this by applying DNA barcoding to resolve the structure of a food web. Our team consisting of researchers from Finland and Canada chose to focus on one of the simplest food webs on Earth: the moths and butterflies of Northeast Greenland, as attacked by parasitic wasps and flies. When the traditional technique of rearing hosts was supplemented with DNA-barcoding techniques, every measure of food web structure changed. We found three times as many interactions between species as before. Most types of predators proved less specialized and most types of prey were attacked by many more predators than assumed. Thus, the full web turned out to be more tightly knit than ever thought.

By designing primers to attach to the DNA of only predators or prey, we were able to selectively amplify and sequence the DNA of immature predators from within their prey, and the remains of the larval meal (prey) from the stomachs of adult predators. By then comparing the sequences to a reference library of DNA barcodes of all species in the region, we could determine exactly who had attacked whom.

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Sympistis nigrita ssp. zetterstedtii



Cryptus arcticus



DNA barcodes for two interacting species of the High Arctic food web. Image credit: Gergely Várkonyi.

Dissecting the Food Webs of the Far North -

Continued from previous page



A landscape from Zackenberg valley, Northeast Greenland.
Image credit: Gergely Várkonyi.

This approach also allowed us to retrace the life history of otherwise hidden predators. As larvae, some of the predators attack their prey when they are hidden in the ground or vegetation. By instead looking for prey remains in the guts of the more easily-detectable adult predators, we were able to establish the importance of these links.

To understand just how much the method changed their perception of their target web, we compared variation among different techniques to variation among food webs previously described for different parts of the world. Strikingly, the web structure varied manifold more among the techniques than among localities from the UK to Japan.

The still-most exciting aspect of the study is the vistas opened by the findings. While barcoding was applied to reconstruct the make-up of one of the simplest food webs on the globe, it completely revamped our view on how this web was structured. Now we can just imagine what will happen when we employ this approach in other settings.

Written by: Tomas Roslin and Helena Wirta

Quagga Mussel Invasion of Western Europe:

Phylogeography, population genetics and potential impact assessment

Biofouling invaders constitute one of the major threats to freshwater biodiversity not only because they have an impact on both aquatic ecosystems and biodiversity but also because they negatively influence industrial activities. The best-known example is the invasion of Western Europe and North America by the zebra mussel (*Dreissena polymorpha*, Pallas 1771). In the meantime, a second dreissenid species, the quagga mussel (*Dreissena rostriformis bugensis*, Andrusov 1897) recently became invasive in both the Old and New World with a first observation in Western Europe in the Hollands Diep in 2006.

We investigated the invasion of *D.r. bugensis* in the Meuse River (Belgium). By using both mitochondrial and microsatellite markers, we analyzed the invasion pathway of the species in Western Europe through a global phylogeography study. Finally, we assessed the impacts of the two invasive *Dreissena* species by determining densities, native mussel perturbation, filtration rate, and the ecological links between *Dreissena* and other macro-invertebrates in the major European Rivers.

Contrary to predicted invasion patterns, the quagga mussel was found in the Dutch, Belgian and French sections of the Meuse River suggesting a rapid colonization since its arrival. **Our study** also demonstrated that even if *Dreissena* specialists discriminate adults of the different species based on shell features, this task remains difficult for managers. Therefore, we proposed the use of the COI mitochondrial gene as a barcode to discriminate the zebra mussel from the quagga mussel. Moreover, a restriction fragment length polymorphism (RFLP) analysis on the COI gene can be conducted to discriminate both *Dreissena* species without additional sequencing costs. This tool is useful for rapid identification of both adult specimens and larvae in order to detect newly invaded habitats.



We gathered the largest sampling dataset compiled to date for our phylogeographic study of the quagga mussel including 32 populations covering its invasion range in both Europe and North America. Using the COI mitochondrial gene and 10 microsatellite loci, we studied the relationships between geographically distinct populations of the quagga mussel to investigate its invasion pathways. Our results revealed low genetic diversity and low genetic differentiation between populations (with an exception for the Western part of North America) and four distinct historical invasion routes.

Over the last 20 years, our laboratory observed a phytoplankton decline in the Meuse River. In order to determine if there is a link between this phenomenon and the introduction of invasive bivalves, we measured the filtration rates of both invasive Dreissenidae and Corbiculidae species in laboratory facilities. Based on these results and density measurements, we estimated that 99% of the water column of the river is filtered by these invasive species on a river stretch of 100km (flow rate of 50m³/s). Moreover, we measured the infestation rate on native bivalves during a technical maintenance of the Meuse River and estimated that around 40% of the native populations were infested by dreissenids.

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Quagga Mussel Invasion of Western Europe -

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Finally, artificial substrates were placed in May 2012 at five stations in the Meuse River in order to study the life cycle of *Dreissena* and the ecological links between them and other macro-invertebrates in the major European Rivers. Preliminary results indicate high growth rates for both zebra and quagga mussel and high relative abundance (up to 70% of the total number of macro-invertebrates).

This study received financial support from the University of Namur, the European Fisheries Fund and the Walloon Region. Jonathan Marescaux is funded by a Ph.D. grant from the Belgian National Fund for Scientific Research (FRS-FNRS).

Written by: Jonathan Marescaux and Karine Van Doninck

Images by: Jonathan Marescaux



Official Launch of NorBOL:

Meeting highlighted the efforts of NorBOL partners while looking ahead

The official start of the Norwegian Barcode of Life Network (NorBOL) as a national research infrastructure for DNA barcoding in Norway was celebrated on March 10, 2014. Attending the kick-off meeting at the NTNU University Museum in Trondheim were invited guests from the Research Council of Norway, the Norwegian Biodiversity Information Centre, the Norwegian Environment Agency, and Norwegian universities and research institutes. The meeting was a highly enjoyable event with presentations of DNA barcoding, NorBOL and iBOL and with speakers acknowledging the effort that NorBOL partners have made to make the project a reality. In particular the international aspects of the DNA barcoding enterprise through CBOL and iBOL were highlighted. The NorBOL partners now look forward to the work ahead and aim to have 20,000 species barcoded by 2018. For more information on NorBOL, please visit www.norbol.org.

Written by: Torbjørn Ekrem

Images by: Åge Hojem, NTNU Vitenskapsmuseet



Barcoding Spiders of Churchill, Manitoba:

DNA barcodes and morphology reveal high species diversity and new Canadian records

Arctic ecosystems, especially those near transition zones, are expected to be strongly impacted by climate change. Because it is positioned on the ecotone between tundra and boreal forest, the Churchill area is a strategic locality for the analysis of shifts in faunal composition. This fact has motivated the effort to develop a comprehensive biodiversity inventory for the Churchill region by coupling DNA barcoding with morphological studies. **Our recent study** represents one element of this effort by focusing on the spider fauna at Churchill.

Among 2704 analyzed spiders, 198 species were detected representing 14 families and 98 genera, tripling the count for the Churchill region. Estimates of overall diversity suggest that another 10–20 species are awaiting detection. Most species displayed little intraspecific sequence variation (maximum <1%) in the barcode region of the cytochrome *c* oxidase subunit I (COI) gene, but four species showed considerably higher values (maximum = 4.1–6.2%), suggesting cryptic species. All recognized species possessed a distinct haplotype array at COI with nearest-neighbour interspecific distances averaging 8.6%. Three species new to Canada were detected: *Robertus lyrifer* (fam. Theridiidae or cobweb weavers),

Baryphyma trifrons, and *Satilatlas monticola* (fam. Linyphiidae or dwarf weavers). The first two species may represent human-mediated introductions linked to the port in Churchill, but the third species represents a range extension from the USA. The first description of a female of *Satilatlas monticola* was presented. As well, one new species of genus *Alopecosa* (fam. Lycosidae or wolf spiders) was recognized.

Our study provides the first comprehensive DNA barcode reference library for the spider fauna of any region. Only a few cryptic species of spiders were detected, a result contrasting with the prevalence of undescribed species in several other terrestrial arthropod groups in the Churchill region. Because most sequence clusters at COI corresponded with a named taxon (97.5%), DNA barcoding reliably identifies spiders in the Churchill fauna. The strong morphological/molecular

correspondence indicates that prior morphological studies have been effective in species recognition in spiders. The capacity of DNA barcoding to enable the identification of otherwise taxonomically ambiguous specimens (juveniles, females) also represents a major advance for future monitoring efforts on the spiders.



Collecting invertebrates at Ramsay Creek, Churchill (2009).
Image credit: Masha Kuzmina



Female and male dorsal view of new wolf spider species of the genus *Alopecosa*.

Written by: Gergin Blagoev

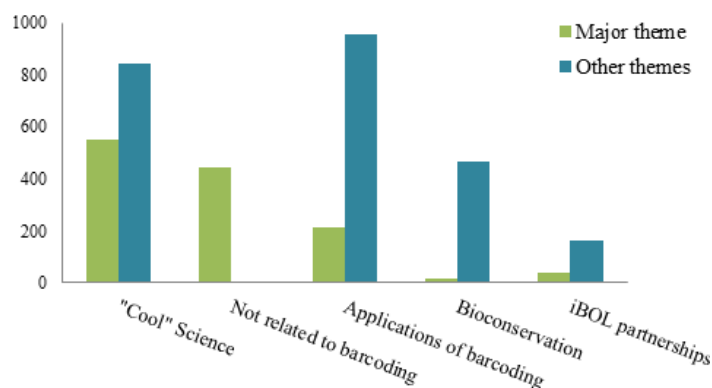
The Public Face of DNA Barcoding:

How iBOL and DNA barcoding are represented in international newspapers

A key goal of the International Barcode of Life (iBOL) Project is to communicate about the science and utility of barcoding technology, including applications to biodiversity research, customs control, and consumer protection. The Project also involves an international collaboration of nearly 30 countries covering all inhabited continents. Such international collaborations raise significant policy challenges, especially because iBOL utilizes genetic resources. Biodiversity rich countries are concerned about the movement of genetic resources to developed countries with research capacity. The utilization of genetic resources in a manner that does not exploit developing countries and provides for the equitable sharing of benefits is central to the operation of the *Convention on Biological Diversity* and the associated *Nagoya Protocol on Access and Benefit Sharing*.

We therefore examined not only how the science and utility of barcoding are communicated in global print media (2003-2012), but also coverage of policy issues raised by barcoding's use of genetic resources. Despite the rise of the internet, the print media remain an important source of information about science and technology, and often set the agenda for news items that later circulate through newer communications platforms.

We found 1266 unique English language newspaper articles on iBOL or DNA barcoding. For each article, we analysed the description of barcoding, the themes, and whether or not it discussed policy issues. Most articles were short, "breaking news" reports (87%) in developed country newspapers (80%). The major theme, identified by its prominent placement at the start of the article, was the "cool" science of barcoding (43%). Unfortunately, as is a common complaint with science journalism, the articles failed to explain the science in a manner that would enable public understanding: only 9% met our criteria of a full scientific description, including species identification based on short DNA sequences compared to a reference barcoding database.



The number of times a topic was a major theme (appeared first in the article) and a minor theme (appeared anywhere in an article).

One positive finding of our study was the number of articles (76%) that mentioned applications for barcoding. However, given the major focus on barcoding as a tool for biodiversity research and monitoring, it was surprising that bioconservation was only mentioned in 37% of articles. Indeed, the important policy issues raised by barcoding were largely neglected: the *Convention on Biological Diversity* was mentioned in only 1% of articles and the *Nagoya Protocol* in two articles. The controversial topic of biopiracy or bioprospecting, depending on whether this activity is characterised negatively or positively, was mentioned only six times.

In conclusion, the average reader of print media would learn that DNA barcoding exists and has some useful applications, but not how the technology works. In focusing on regulatory utility, media articles have lost sight of the core value of barcoding as a tool for biodiversity research. The opportunity to use the "cool" science of barcoding as a door to open discussion about the critical conservation issues faced by our planet has not been fully realised, and there is no engagement of the media with the complex policy discussions over essential international biodiversity research initiatives.

Written by: Janis Geary, Emma Camicioli, and Tania Bubela

Barcoding in a Community Lab:

Public engagement a key objective of the Alaska Barcode Project

An important part of science literacy efforts is to create awareness of the positive impact of scientific advances. DNA-based technologies are often viewed with suspicion, and are frequently represented in the public eye by GMOs and loss of genomic privacy. Citizen science projects appear to be particularly effective in the education of adults, especially those that include hands-on participation in field work or wet work. A worthy, shared goal is a powerful motivator, and a deeper understanding of the technology is gained in the course of the group effort.

Genspace is a nonprofit community laboratory in Brooklyn, NY. It was founded in 2009 to fill the public need for an open, non-affiliated lab space with a low barrier to entry in terms of education and price point. Its mission is to promote learning and innovation in the sciences by providing mentorship and a fully-equipped BSL1 lab available for citizen science projects.

In 2012 we began what was to become known as the Alaska Barcode Project, an effort to engage the public by DNA barcoding plants of the Alaskan tundra. The contrast between the hardiness of plants capable of surviving Alaskan winters and their fragility as part of the threatened Alaskan environment make this survey particularly appealing. Initial funding for the project was through a small grant from Autodesk.

To collect the plants, a small expedition team journeyed by bush plane into the backcountry near the town of McCarthy, which is located proximal to Wrangell-St. Elias National Park in South Central Alaska. Approximately 300 whole plant specimens were collected from a high glacial river valley, pressed, and brought back to Brooklyn for barcoding. Dr. Damon Little of the New York Botanical Garden, an Alaskan himself, tutored us in proper plant collection methodology and agreed to aid in the traditional identification of the specimens.



Plants collected by Ellen Jorgensen in Alaska are then DNA barcoded by the public in a community laboratory.

DNA barcoding is carried out as a social activity during Open Nights at Genspace, where the public is invited to extract DNA and perform PCR amplification prior to sending the samples out for sequence analysis. We announce the events on our website and through Meetup.com, where the barcoding activity consistently earns a five-star rating from participants. Since barcoding is not technically demanding, it is a perfect introduction to DNA science for adult non-scientists. It also fits well into an evening session's timeframe. We have hosted more than a half-dozen of these events, and received a universally positive response from participants. There is a feeling of ownership of the project. Many people return to prepare additional samples or to see the sequence data from the samples that they processed. Although we have just started the sequencing phase of the project, we have already generated novel barcode sequences for submission. It's easy to grasp the implications of having a simple way of identifying and cataloging species from threatened environments.

A successful crowdfunding campaign has raised additional funds for the project. This August we will return to Alaska and survey an additional site, collecting a second round of plants. In addition, we plan to partner with Alaskan native communities and the National Park Service to expand our barcoding efforts.

Written by: Ellen Jorgensen

Barcoding Mushrooms in a Biodiversity Hotspot:

Citizen science in action

California has more species, more endemic species, and more rare and endangered species than any other state in the United States, and San Diego County in particular is an internationally recognized biodiversity hotspot. Due to varied topography and a Mediterranean climate, it represents the taxa-rich boundary between the southern range limit of many northern species and the northern limit of some southern species. But human development has created tremendous habitat loss, so local citizen scientists are working to scientifically document this diversity before it is lost.

Volunteers from the San Diego Mycological Society (SDMS) for example, began vouchering mushroom specimens gathered during their forays in the SD Herbarium at the San Diego Natural History Museum, with the goal of eventually creating a synoptic collection of mushrooms for the county. Inspired by participation in a recent workshop on barcoding held at the University of California San Diego, some SDMS members quickly realized that they could take what they were already doing to the next level, by also taking fresh tissue samples from the specimens that could be subsequently used for DNA barcoding.

This was seen as a win-win for the SDMS, because it provided the opportunity to have fun while generating some useful scientific data. The best part is that everyone who wants to participate can contribute to the project in their own way. Members who are most interested in mushroom hunting can use their skills to find and bring in the specimens, those who enjoy identification can key them out, photographers can document the specimens, while others with a more scientific bent can take charge of recording the field data, taking the tissue samples, and vouchering the specimens at the museum. Afterward, everyone can divide up whatever is left over and still enjoy their favorite aspect of mushrooming - cooking, eating, or expressing their creativity by using them for arts and crafts!



Each February, SDMS holds a popular, free public education event called the Fungus Fair in beautiful Balboa Park, to educate people and share their enthusiasm for mycology. Members participate in a foray to collect specimens for display, and this year they worked with iBOL so that they could take tissue samples from the mushrooms and save them for future barcoding before the specimens were deposited in the permanent collection in the museum's herbarium.

Going forward, SDMS will be promoting their new mushroom barcoding project to help increase public awareness about barcoding, local conservation issues, and the world-wide significance of San Diego's biodiversity. In addition, this interesting pilot project will strengthen the efforts of the San Diego Citizen Science Network to encourage and inspire citizen science projects locally. Most importantly, the barcoding data that are generated will be added to BOLD so that it can be shared with, and used by the scientific community.



Written by and images by: Mary Ann Hawke

Identifying Birds and Saving Lives:

DNA barcoding revolutionizes birdstrike investigations

On January 15, 2009, US Airways Flight 1549 from New York to Seattle struck a flock of birds during its initial climb out, lost engine power, and ditched in the Hudson River off midtown Manhattan with no loss of life.

In the United States alone, around 7000 bird–aircraft collisions (birdstrikes) are reported annually. Most of these strikes occur during takeoff or landing of the flight, and it is during these flight phases that aircrafts experience the highest risk of substantial damage after colliding with birds. Worldwide, birdstrikes are estimated to cost the commercial airline industry a minimum of \$1.1 billion per year and have resulted in more than 210 aircrafts destroyed and 229 deaths since 1988.

To reduce the risk of a strike, airport authorities try managing habitats on the airfield to make them less attractive for birds or by deterring them in different ways. They have a variety of noise-making pyrotechnics that startle birds but also dogs and even predatory birds are used to scare away birds. Vancouver Airport for example has falcons, a bald eagle and dogs to chase geese and scare away a variety of shorebirds. The knowledge of whether bird species are migratory or not can be crucial to prevent future birdstrikes. If birds belong to a species permanently living near an airport, reducing the population could be the appropriate management technique, while if they are migratory birds, using radar to detect migratory flocks of birds and make adjustments to flight plans could help minimize the potential for future birdstrikes in the area.

All of these measures have in common that they require some knowledge about the species identity of the birds that could pose a risk to air traffic. Unfortunately, there is not much left of a bird once it hits a plane or gets into a turbine. In the past, species often could only be identified by a hopefully intact feather but this method is by no means fully reliable and, because it requires expertise in feather microstructure and access to a large reference

collection of downy feather microslides for accurate comparisons, there are only a few experts in the world who are able to conduct such specific identifications. The invention of DNA barcoding represented a huge step forward and changed the approach quite a bit over the last few years.



Image credit: Michael E. Baird

About 48% of the known birds have been barcoded to date, providing a large resource that can help to identify bird remains after a collision with an aircraft. As a consequence, the Biodiversity Institute of Ontario (BIO) receives samples from airport authorities and institutions they work with on a regular basis. One recent case involved bird remains sent from the Vancouver

airport. DNA barcodes matched sequences on the Barcode of Life Datasystems (BOLD) belonging to the Great Blue Heron (*Ardea herodias*), a rather large species that occasionally is involved in birdstrikes which often cause extensive engine damage. Another sample sent in from a different source had a little surprise in stock. The remains were identified as a mixture with DNA sources from two different species, one being a mourning dove (*Zenaida macroura*) which is not uncommon but the other DNA source matched the seminoe bat (*Lasiurus seminolus*). The little bat likely would not cause any damage during a collision and therefore does not represent a concern. Collisions between bats and aircraft are not uncommon and there are a few cases where they have been used for research in order to determine flight altitudes of bats (Hoary bat, *Lasiurus cinereus*; flying foxes, Pteropodidae).

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Identifying Birds and Saving Lives -

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DNA barcoding played a central role in the investigation of the birdstrike involving US Airways Flight 1549 back in 2009. BIO analyzed samples retrieved from the aircraft and the DNA barcodes matched to *Branta canadensis*, the Canada goose. Additional stable hydrogen isotope analysis done at the Smithsonian Migratory Bird Center in Washington DC determined that the geese belonged to a migratory population.

The addition of DNA barcoding has certainly improved the ability to make species-level identifications in birdstrike incidents and it could also be useful for conservation by aiding our understanding of flight patterns of migratory bird species (and those of flying mammals) of concern.

Written by: Dirk Steinke



Austrian Barcode of Life (ABOL) Receives Support:

Pilot project will focus on four organismal groups while building infrastructure

In support of the ABOL initiative, Minister Dr. Reinhold Mitterlehner recently announced that the University of Veterinary Medicine, Vienna (VetMed) and the Natural History Museum, Vienna (NHM) will be leading a pilot project, financed through structural funds for universities. Through extensive collaboration with other Austrian institutions, the project aims to collect DNA barcode data for four organismal groups: vertebrates, molluscs, lepidopterans, and parasitic worms.

By initiating the formation of infrastructure required to establish national databases and aggregate existing data, this project will pave the way for the larger goal of the ABOL project: cataloging all of Austria's fauna, flora, and fungi using DNA barcoding. A meeting will be held in Fall 2014 to discuss the structure of the future ABOL project.

National DNA Barcoding Project Launches in Peru:

PeBOL set to raise awareness and build national DNA barcoding capacity in a biodiverse country

Peru ranks among the world's top 15 biodiversity-rich countries. Its unique abundance of biological and mineral resources holds great opportunities for economic prosperity. However, high levels of poverty and limited planning in urbanization and agricultural development pose serious challenges. Although comprehensive legislative measures have been put in place at the national level to ensure sustainable use of Peru's natural resources, their implementation is hampered by scarcity of information. These challenges are particularly strong with biodiversity which underpins the health, resilience and productive capacity of natural ecosystems. However, unlike land cover, it cannot be assessed remotely without intensive sampling.

In response to this challenge, Peruvian and Canadian experts have partnered to launch a nation-wide project aimed at building Peru's capacity to survey and use its biodiversity through DNA barcoding. This was made possible thanks to a \$360K contract awarded to the Biodiversity Institute of Ontario, University of Guelph. The project is funded by Canada's Department of Foreign Aid, Trade and Development (DFATD) and administered by the Conference Board of Canada under the Canada-Americas Trade-related Technical

Assistance (CATRTA) Program. The overarching goal of this program is to advance development and poverty reduction through maximizing opportunities of free trade agreements in Latin American countries.

This two-year CATRTA project titled "DNA Barcoding to Support Biodiversity Conservation, Sustainable Harvesting and Trade in Peru" aims to aid Peru in building its technical knowledge and DNA barcoding capacity. It is jointly executed by the Peruvian Museum of Natural History, San Marcos University (MHN-UNMSM) and the Biodiversity Institute of Ontario, with support from Environment Canada and the Peruvian Ministry of the Environment (MINAM). Led in Peru by the Museum's Director, Betty Millan and Curator, Rina Ramirez, this effort has brought together a strong national expert team representing academic institutions, government agencies and NGOs. Its primary goal is to raise awareness about DNA barcoding among the Peruvian research community, regulatory authorities, private sector companies and the general public. It further aims to build national expertise in this technology through a series of training activities within and outside Peru.

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National DNA Barcoding Project Launches in Peru-

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The project was formally launched with the Peruvian National DNA Barcoding Workshop that took place in Lima on March 11-13, 2014. The workshop started with an introductory session at the Lima Chamber of Commerce that included nearly 100 participants from all over the country. It was followed by a two-day in-depth training session for 40 participants, held at Vista Pacifico conference centre in Asia (vicinity of Lima). In their opening remarks, Javier Verastegui (Peruvian Science and Technology Council – CONCYTEC) and Jose Alvarez Alonso (MINAM) emphasized the importance of DNA barcoding in helping understand, preserve and use Peru's biodiversity.

Immensely valuable insights were provided by keynote speakers Pablo Tubaro (Museo Argentino de Ciencias Naturales, Argentina) and Manuel Elias (El Colegio de la Frontera Sur, Mexico) who shared their experience in successfully launching and coordinating DNA barcoding activities in their respective countries. Julianne Diller (Bavarian State Zoological Collection, Germany) provided an overview of the partnering Peruvian Panguana project and Brenda McAfee (Environment Canada) advised on international agreement frameworks and policy matters related to biodiversity. Further comments on access and benefit sharing policies were made by Manuel Ruiz (The Peruvian Society for Environmental Law - SPDA). Training modules were delivered by Alex Borisenko and Karina Gonzales (Biodiversity Institute of Ontario).



Several productive group discussions focussed on building the roadmap for advancing DNA barcoding in Peru and defining priority areas for its application. The workshop received an overwhelmingly positive response from the participants and received considerable media attention: it was showcased in several interviews on local radio and TV and on the [website of the Peruvian Ministry of Environment](#).

The Peruvian Barcode of Life website has been launched in conjunction with the Workshop – <http://pebol.org/>. It will become the central data resource and information exchange platform for the Peruvian DNA barcoding community.

The project will continue with advanced training for ten selected participants via the University of Guelph's Center of Open Learning online DNA barcoding course, six of whom will visit the Canadian Centre for DNA Barcoding in Guelph in the summer of 2014 for hands-on training in specimen processing and analytical protocols. These experts will be ideally positioned to lead and coordinate the advancement of future DNA barcoding activities in Peru.

This effort will result in a collaborative strategy that will set the stage for improved biosecurity and regulatory compliance through adopting DNA barcoding practices by Peruvian stakeholders. It will lead to strengthened Canada-Peru trade relationships under the Free Trade Agreement while advancing the objectives of the Canada-Peru Agreement on Environment. It will also help identify priority areas of research focus and applications for DNA barcoding in Peru, in accordance with local priorities. By extension, it will boost Peru's opportunities in economic growth and trade in areas directly and indirectly related to biological diversity and environment (e.g., extractive industries, food security, and human health).

Written by: Alex Borisenko

GBOL1 Workshop:

Connecting taxon coordinators and citizen scientists

Since November 2011, the German Barcode of Life (GBOL) project has been operating to genetically characterize and inventory the fauna, flora and fungi of Germany. GBOL is a national consortium of 17 natural history museums and biodiversity research institutions, 47 professional scientists and more than 200 qualified citizen scientists. The GBOL partners provide their professional taxonomic expertise and existing infrastructure (e.g. dry and wet collections/biobanks, databases, bioinformatics platforms and laboratories) to establish a comprehensive genetic library of biodiversity. The Zoological Research Museum Alexander Koenig (ZFMK) in Bonn, Germany coordinates this national DNA barcoding campaign. On January 24th/25th 2014, taxonomic coordinators of the GBOL1 projects (i.e. fauna of NW-Germany), external taxonomic experts and collectors met for a two-day workshop at the ZFMK.

On the first day, Prof. Dr. Wolfgang Wägele (GBOL spokesman) and Dr. Stephanie Pietsch (GBOL coordinator) started with an overview of the GBOL project and interesting aspects and applications of DNA barcoding in general. Afterwards, the taxonomic coordinators provided insights into the status quo of their individual projects. By the end of 2013, the GBOL database listed DNA barcodes for more than 8000 species (11% of known species in Germany) – with many samples awaiting processing in the near future. As a consequence, the ZFMK (and the whole GBOL community) received the award “Ort des Fortschritts in NRW” (“Place of Progress in NRW”) from the North Rhine-Westphalian (NRW) Minister of Science Svenja Schulze in October 2013. The first workshop day was closed by a practical evening session introducing DNA barcoding sequence analysis.



On Saturday morning, all participants were invited to catch a glimpse behind the museum scenes. The ZFMK staff gave a tour through their GBOL facilities. The whole barcode-pipeline from sample arrival to the published database record was demonstrated. Subsequently, coordinators, experts and collectors started their group work discussing individual barcoding datasets, future collecting and outreach strategies.

The high attendance as well as the positive feedback from 50 participants made the GBOL1 workshop a great success both for GBOL taxonomists and for external taxonomic experts.



Written by: Alexander M. Weigand and Stephanie Pietsch

Trends in DNA Barcoding Publications:

A look at a decade's worth of publications

Usually this page contains a top ten list of the annual publications but such a list for 2014 would not be very useful so early in the year. For this reason we decided to present a top ten list only in the last 2014 issue in December. Meanwhile this page will provide you with some statistics on DNA barcoding publications in general which we hope you find useful. We will start with a general figure showing the trend over the first decade of DNA barcoding.

Since the publication of the famous Hebert *et al.* paper in 2003, 2822 publications involving DNA

barcoding have been produced. The figure below shows the steady increase over the last ten years. In the early years DNA barcoding was heavily criticized but only 35 publications (1.2%) actually reflect that. One of the main criticisms was that it would not help to improve current taxonomic knowledge. A more detailed look at all 2822 publications revealed that almost 10% of them are strictly related to taxonomy (new descriptions, revisions, discoveries confirmed with morphology).

Written by: Dirk Steinke

DNA barcoding publications

